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Randomized, double-blind, placebo-controlled clinical trial on the usefulness of probiotic *Lactobacillus reuteri* in bismuth-containing quadruple eradication therapy for infection with *Helicobacter pylori*

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ABSTRACT

Introduction: the primary goal of this study was to compare gastrointestinal symptom reduction in patients on bismuth-containing quadruple eradication therapy supplemented with *Lactobacillus reuteri* strains (DSM 17938 and ATCC PTA 6475) or placebo.

Materials and methods: this was a randomized, double-blind, parallel-arm, placebo-controlled clinical trial. Patients received a first-line eradication regimen based on bismuth subcitrate potassium, metronidazole, tetracycline hydrochloride (three-in-one capsules) and omeprazole 40 mg twice a day for ten days, plus a probiotic or placebo

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tablet for 30 days. During follow-up, gastrointestinal symptoms were assessed using an evaluation scale (GSRS), and adverse events were collected at 0, 14, 28 and 56 days.

Results: a total of 80 patients were included from February 2018 to May 2019 at a single site. Eradication therapy was effective in 85 % of patients, with no differences between treatment arms. In the group receiving the probiotic, abdominal pain decreased in 42 % of patients, compared with 19 % in the control group (OR: 0.27; CI, 0.13-0.58; p < 0.001), and abdominal distension decreased in 25 % versus 17 % in the control group (OR: 0.24; IC, 0.19-0.84; p < 0.001);

Conclusions: treatment with *L. reuteri* only reduced abdominal pain and distension. Further studies are needed to establish the role of probiotics as adjuvant therapy in *H. pylori* eradication.

Keywords: *Helicobacter*. *Lactobacillus*. Eradication. Probiotics. Bismuth. Quadruple.

INTRODUCTION

Infection with *H. pylori* is a well-known risk factor for gastric cancer, which is the second leading cause of cancer-related death worldwide (1). Although effective therapies are available (2), the eradication rate remains highly variable among regions and countries. Factors associated with eradication failure also include antibiotic resistance and early discontinuation, which make newer strategies necessary (3)

Different reported results suggest that probiotics may be added to eradication therapy to improve efficacy and adverse events (4-7). This recommendation is also included in the Maastrich IV/Florence consensus report, which points out the usefulness of some probiotics and prebiotics for the management of infections caused by *H. pylori.* Probiotics show promising results as therapy adjuvants, and reduce associated adverse events (8,9).

Among a wide variety of probiotics, some strains of *Lactobacillus* have been found to inhibit *H. pylori* growth in vitro, and to block its adherence to gastric mucosal cells (10). Evidence has been reported on the use of *Lactobacillus reuteri* strains, in combination

with triple antibiotic therapy for *H. pylori* eradication, but it is rare regarding quadruple therapies (3).

The primary aim of this clinical trial was to compare the reduction in gastrointestinal symptoms, as measured by the Gastrointestinal Symptoms Rating Scale (GSRS), in patients with bismuth-containing quadruple eradication therapy plus *L. reuteri* strains versus placebo.

MATERIALS AND METHODS

Study design

This study was a randomized, double-blind, parallel-arm, placebo-controlled clinical trial. The study was conducted in accordance with GCP E6 (R2) EMA/CHMP/ICH/135/1995. All study documents were reviewed and approved by the local Ethics Committee under code C.I. 2017/337, and by the "Agencia Española del Medicamento y Productos Sanitarios" (AEMPS), including the study protocol registered with the name BISMUGAS-2017-04.

Participants

All participants aged between 18 and 65 years were selected among the patients with confirmed *H. pylori* infection who visited the gastroenterology department at the "Hospital Virgen Macarena" (Seville, Spain) over 12 months. The diagnosis with infection was confirmed using any available technique (13C-urea breath test, histology, *H. pylori* stool antigen test). Prior to any intervention, all of the enrolled participants gave their informed consent to participate in writing. Patients with previous eradication, use of non-steroidal anti-inflammatory drugs (NSAIDs) within 3 weeks, prior therapy with probiotics within 4 weeks or previous use of antibiotics within 2 weeks were excluded. Pregnant women were also excluded. During the trial, treatment with other probiotics, antibiotics, anti-H2 agents or NSAIDs was not allowed.

Intervention

At the first visit, patients received a complete eradication therapy regimen based on bismuth subcitrate potassium, metronidazole and tetracycline hydrochloride (three-in-one capsules: 140 mg/125 mg/125 mg), three capsules four times a day, plus omeprazole 40 mg twice a day for ten days. Patients also received a chewable tablet with *L. reuteri* in the experimental arm, or maltodextrin in the control arm for 30 days.

Efficacy endpoint

Subjects were required to complete four visits for follow-up completion at a maximum of 56 days since the first visit. The second visit took place upon completion of eradication therapy at 14 ± 3 days; the third visit was scheduled after finishing therapy with probiotics at 28 ± 3 days and the last follow-up visit occurred at 56 ± 3 days from treatment onset. Gastrointestinal symptoms were assessed during all visits using the GSRS score (Svedlund et al., 1988) and adverse events were collected by the attending physicians. Patients also received a diary to assess their adherence to treatment as well as minor side effects. At the end of follow-up, *H. pylori* eradication was confirmed by means of a 13C-urea breath test.

Sample size

Sample size was estimated considering that the experimental group would reduce symptom scale scores by 25 % versus the control group. In the control group, the mean symptom score was estimated to be 6.8 points by previous testing, with a standard deviation of 3-1 units. Calculations assumed an alpha error of 5 %, a power of 90 % and percent losses of 10 %. The final sample size was estimated as 40 subjects per treatment arm, for 80 patients in total.

Randomization

The Delos Clinical contract research organization (CRO) assigned an anonymous code to each enrollee by order of inclusion, which allowed to protect personal data confidentiality. Using this code, each patient was randomized to the experimental group or placebo group in a 1:1 fashion, following a predefined randomization list.

Blinding

To ensure blinding, both treatments were prepared and labeled identically. Patients, researchers and statisticians remained unaware of trial arms until the end of the statistical analysis.

Statistical methods

Categorical variables were described using absolute and relative frequency tables.

For the comparison between both treatment arms, the normal distribution assumption was not met, hence a Mann-Whitney non-parametric U-test for independent samples was performed. All statistical analyses were performed using the RStudio, version 1.3.4 software program, assuming an alpha and beta error of 5 % and 20 %, respectively.

Quality of data

The study was audited by Delos Clinical, a CRO independent of the sponsor and research team, as established in the monitoring plan. Compliance with good practice standards was verified, as well as with all ethical and legal requirements in force.

RESULTS

A total of 80 patients were included from February 2018 to May 2019; of these, 68 (85 %) patients completed follow-up. Reasons for early discontinuation included loss to follow-up (13.7 %) and an adverse event in one case (1.2 %) (Fig. 1).

The demographics and comorbidities of the population are listed in table 1. No differences were found between treatment groups except for a greater proportion of gastrointestinal comorbidities in the control group (7.5 % vs 27.5 %; p = 0.039). These gastrointestinal comorbidities mainly included Crohn's disease and ulcerative colitis among those who received placebo.

Overall adherence to eradication therapy was 77.5 %, 80 % in the control group and 75 % in the probiotic group. In the arm with probiotic therapy, one patient completed only five days of eradication treatment and the median number of days was 10 in this group. In the control group, all patients completed at least 9 days of tretament and the median number of days was also 10. Adherence to the study treatment was higher

than 89 % (87.1 % in the probiotic group and 90 % in the control group). There were no differences in treatment compliance between the groups.

Eradication therapy was effective in 85 % of patients. There were no differences in eradication rate between treatment arms (80 % vs 90 %; p = 0.228). Both treatment arms achieved a net reduction of 6 points in the GSRS score at the end of treatment (p < 0.001). The maximum reduction in GSRS score that was observed occurred within the initial 14 days of treatment; afterwards, reductions continued but were less pronounced (Fig. 2). In the probiotic group, patients achieved reduced scores in all GSRS subscales except for bowel dysfunction syndrome. However, those in the control group only showed reductions in the indigestion syndrome subscale (Table 2). There was no differences in overall or subscale GSRS scores between the probiotic and control group at therapy completion.

We compared the differences observed pre- and post-treatment in both study arms. In the probiotic arm, there was an improvement in 7 of the 15 symptoms included in the GSRS instrument, whereas in the control arm, there was improved scores for 6 of all 15 symptoms. Increased flatulence, heartburn, acid regurgitation and epigastric pulling improved in both arms (Table 3).

Figure 3A shows the percentage of patients with each symptom before and after treatment, and figure 3B shows the magnitude of the reduction in each treatment arm. Abdominal pain diminished for 42 % of patients in the probiotic group, compared with 19 % in the control group (odds ratio (OR): 0.27 (CI, 0.13-0.58; p < 0.001)); abdominal distension and belching decreased by 25 % and 26 %, respectively, in the probiotic group compared with 17 % in the control group (OR: 0,24 (CI, 0.19-0.84; p < 0.001) and OR: 0.6 (CI, 0.29-1.28; p = 0.266)).

A total of 20 adverse events were reported in 16 patients, with no differences between treatment arms. All adverse events were moderate or mild in severity except in one patient in the control group, who abandoned the study because of skin rash, glossitis and vomiting.

DISCUSSION

Infection with *H. pylori* is one of the most prevalent infections worldwide (8). Analyzing the effectiveness of eradication therapy and looking for alternatives that may improve it are key actions to reduce the prevalence of this infection. In our study, eradication rates were 85 %, similar to previous studies of bismuth-containing quadruple therapy (12). However, regardless of eradication rates, these treatments induce adverse events that limit compliance with this eradication protocol (12). In this clinical trial, there were no differences in eradication or adverse event development rates between the probiotic and the placebo group. These results match those reported by a similar trial (13).

As per the reductions seen in gastric subscales, patients supplemented with probiotics experience improvement in all gastric subscales but not in the intestinal subscale. Symptoms improve for all patients, particularly with the eradication protocol, and patients on probiotics achieve a greater reduction in abdominal pain and distension versus placebo. Regarding safety, in view of the results obtained, this treatment can be deemed safe, but caution should always be exercised just in case of allergic reactions to any excipient.

In this study, we encountered three design-related limitations. First, randomization allowed us to compare groups with initially identical scores in the GSRS tool. However, when assessing subscales, subjects in the probiotic arm obtained higher scores for abdominal distension syndrome and dyspeptic syndrome, and therefore outcomes may be undervalued. Secondly, a more restrictive selection of patients would have been desirable. By improving inclusion criteria, further studies may avoid the confounding bias introduced by patients with Crohn's disease, given its controversial association with *H. pylori* infection (14). Furthermore, it will be necessary to select patients with a more specific profile, for instance, dyspeptic subjects, where greater symptom reductions may be achieved by probiotics. Finally, follow-up was decided based on GSRS sensitivity (15), which may be insufficient to measure beneficial probiotic effects given the high stability of the gut microbiota in humans (14), which might explain the lack of improvement in the intestinal subscale.

We conclude that treatment with *L. reuteri* provides a reduction in all gastric subscales, particularly in abdominal pain and distension symptoms. Further studies are needed in

subjects with specific symptom profiles over a prolonged follow-up period in order to establish the actual contribution of adjuvant therapy with a probiotic.

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Table 1. Summary table

	Total n = 80	**Probiotic n = 40**	**Control n = 40**	**p-value**
Demographic data				
Male gender, n (%)	38 (47.5)	16 (40)	22 (55)	0.263
Median age in years (IQR)	50.50 (17.0)	49.40 (17.0)	51.6 (18.0)	0.776
Comorbidities				
Allergies	12 (15.0)	6 (15.0)	6 (15.0)	0.999
Head, eyes, ENT	4 (5.0)	3 (7.5)	1 (2.5)	0.608
Respiratory	6 (7.5)	2 (5.0)	4 (10.0)	0.671
Cardiovascular	23 (28.8)	11 (27.5)	12 (30.0)	0.999
Gastrointestinal	14 (17.5)	3 (7.5)	11 (27.5)	0.039
Genitourinary	1 (1.3)	1 (2.5)	0 (0.0)	0.999
Neurologic, psychiatric	4 (5.0)	1 (2.5)	3 (7.5)	0.608
Hematological, lymphatic	2 (2.5)	2 (5.0)	0 (0.0)	0.474
Dermatological	1 (1.3)	1 (2.5)	0 (0.0)	0.999
Musculo-skeletal	5 (6.3)	4 (10.0)	1 (2.5)	0.355
Surgical history	21 (26.3)	10 (25.0)	11 (27.5)	0.999
Other	9 (11.3)	7 (11.3)	2 (5.0)	0.135

Table 2. Comparison of GSRS scores before and after treatment between probiotic and control therapy

		Probiotic Median (IQR)	**Control group Median (IQR)**	**p**
Total GSRS	Pre Tx.	11 (4)	10 (10.5)	0.218

	Post-Tx.	5 (7.75)*	4 (14)*	0.113
Abdominal pain syndrome	Pre Tx.	1 (1)	0 (1)	< 0.001
	Post-Tx.	0 (1)*	0 (1)	0.613
Dyspeptic syndrome	Pre Tx.	5 (5)	3 (4.75)	< 0.001
	Post-Tx.	2 (2)*	1 (2)	0.083
Indigestion syndrome	Pre Tx.	4.5 (4)	3 (5)	0.138
	Post-Tx.	2 (3)*	1.5 (3)*	0.099
Bowel dysfunction syndrome	Pre Tx.	1 (3)	2 (4)	0.279
	Post-Tx.	1 (3)	2 (4)	0.743

*p < 0.001 for comparison between pre- and post-treatment.

Table 3. Comparison of GSRS symptoms pre- and post-treatment between the experimental and control groups

Scale	**GSRS symptoms**	**Pre-treatment**			**Post-treatment**			**p**
Abdominal pain syndrome	*Abdominal pain*	*Median*	*p25*	*p75*	*Median*	*p25*	*p75*	
	Probiotic	1.00	1.00	2.00	0.00	0.00	1.00	*< 0.001*
	Control	0.00	0.00	1.25	0.00	0.00	1.00	0.1488
Dyspeptic syndrome	*Heartburn*							
	Probiotic	1.00	1.00	2.00	0.00	0.00	1.00	*0.012*
	Control	1.00	1.00	2.00	0.00	0.00	1.00	*0.009*
	Acid regurgitation							
	Probiotic	1.00	0.00	2.00	1.00	0.00	2.00	*0.039*
	Control	0.00	0.00	1.00	0.00	0.00	0.25	*0.010*
	Epigastric pulling							
	Probiotic	1.00	0.50	2.00	0.00	0.00	1.00	*0.002*
	Control	1.00	0.00	2.00	0.00	0.00	0.00	*0.003*
	Nausea and vomiting							
	Probiotic	1.00	0.00	2.00	0.00	0.00	1.00	0.080
	Control	0.00	0.00	1.00	0.00	0.00	0.00	0.525
Indigestion syndrome	*Abdominal distension*							
	Probiotic	2.00	1.00	2.00	1.00	0.00	1.00	*0.008*
	Control	1.00	1.00	2.00	0.00	0.00	1.00	0.052
	Borborygmus							

	Probiotic	1.00	0.00	2.00	0.50	0.00	1.00	0.066
	Control	1.00	0.00	1.25	0.00	0.00	1.00	0.215
	Belching							
	Probiotic	1.00	0.00	2.00	0.00	0.00	1.00	*0.017*
	Control	1.00	0.00	2.00	0.00	0.00	1.00	0.054
	Flatulence							
	Probiotic	1.00	0.50	1.50	1.00	0.00	1.00	*0.028*
	Control	1.00	0.50	2.00	0.00	0.00	1.00	*< 0.001*
Colonic dysfunction syndrome	*Decreased stools*							
	Probiotic	0.00	0.00	0.00	0.00	0.00	0.00	0.510
	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.667
	Increased stools							
	Probiotic	0.00	0.00	0.00	0.00	0.00	1.00	0.410
	Control	0.00	0.00	0.25	0.00	0.00	0.00	*0.030*
	Soft stools							
	Probiotic	0.00	0.00	0.00	0.00	0.00	1.00	0.500
	Control	0.00	0.00	1.00	0.00	0.00	0.00	0.639
	Hard stools							
	Probiotic	0.00	0.00	0.00	0.00	0.00	0.00	0.965
	Control	0.00	0.00	0.00	1.00	0.00	0.00	*0.007*
	Bowel urgency							
	Probiotic	0.00	0.00	1.00	0.00	0.00	1.00	0.650
	Control	0.00	0.00	1.00	0.00	0.00	1.00	0.926
	Incomplete bowel movement							
	Probiotic	0.00	0.00	1.00	0.00	0.00	1.00	0.448
	Control	0.00	0.00	1.00	0.00	0.00	1.00	0.507

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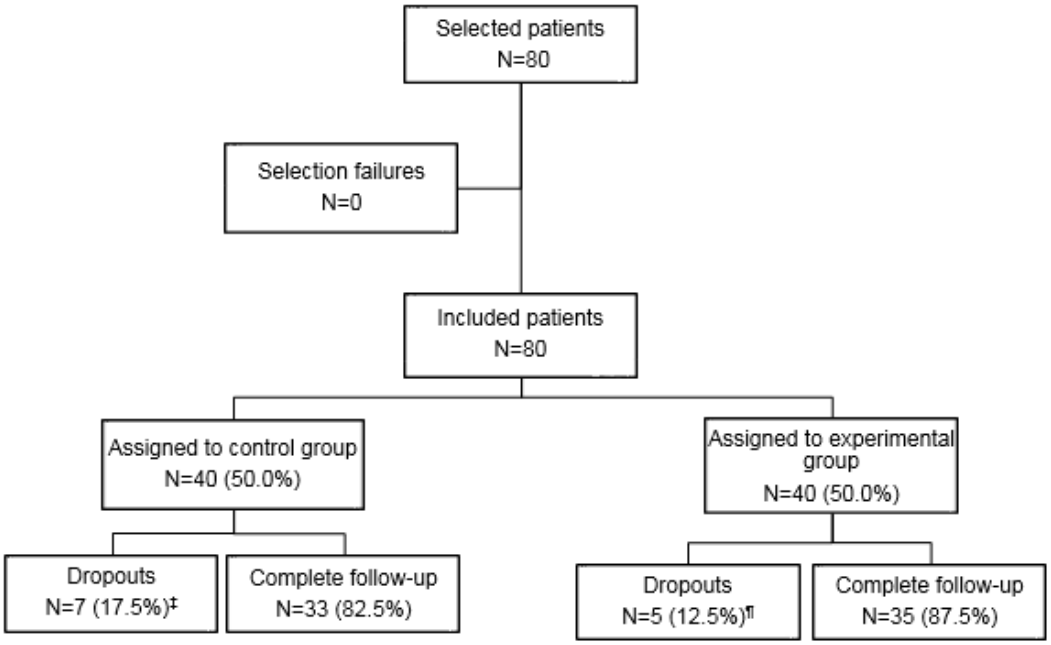

Fig. 1. Patient flow (*six patients (85.72 %) did not attend all appointments, and one patient (14.28 %) could not complete follow-up because of an adverse event; †five patients (100 %) did not attend all appointments.

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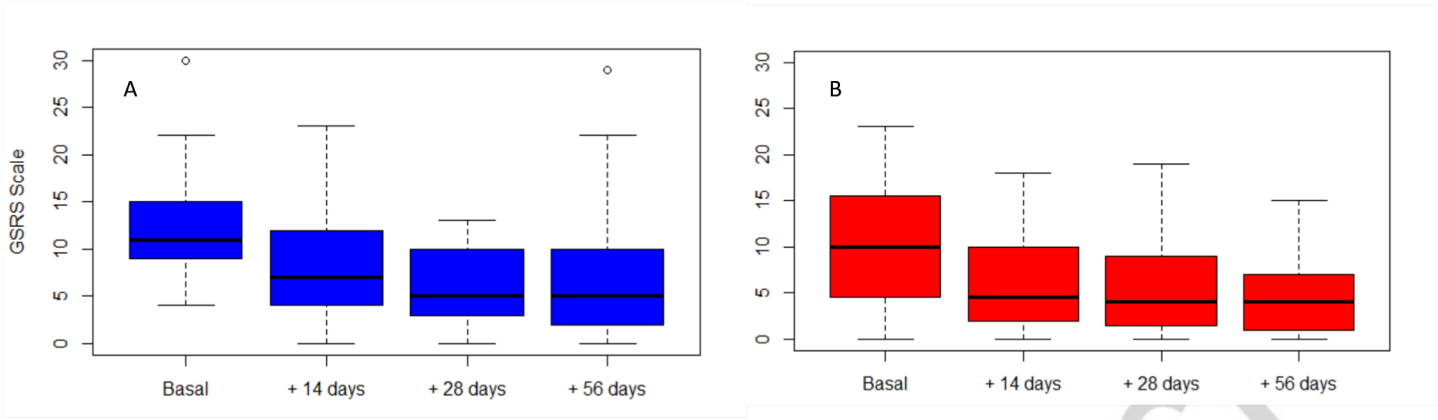
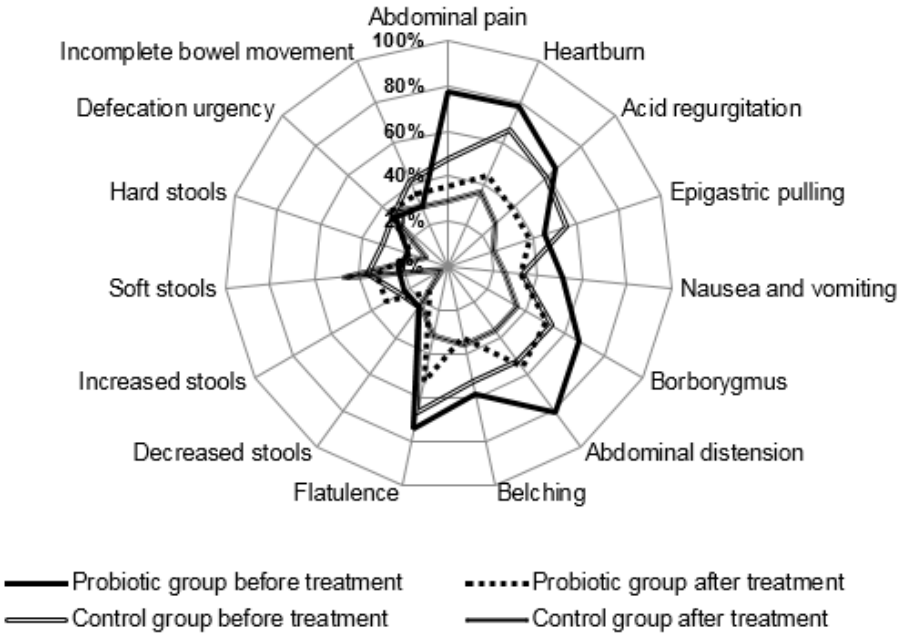


Fig. 2. Evolution of GSRS score during treatment in both study arms. A) Experimental therapy arm. B) Placebo arm.



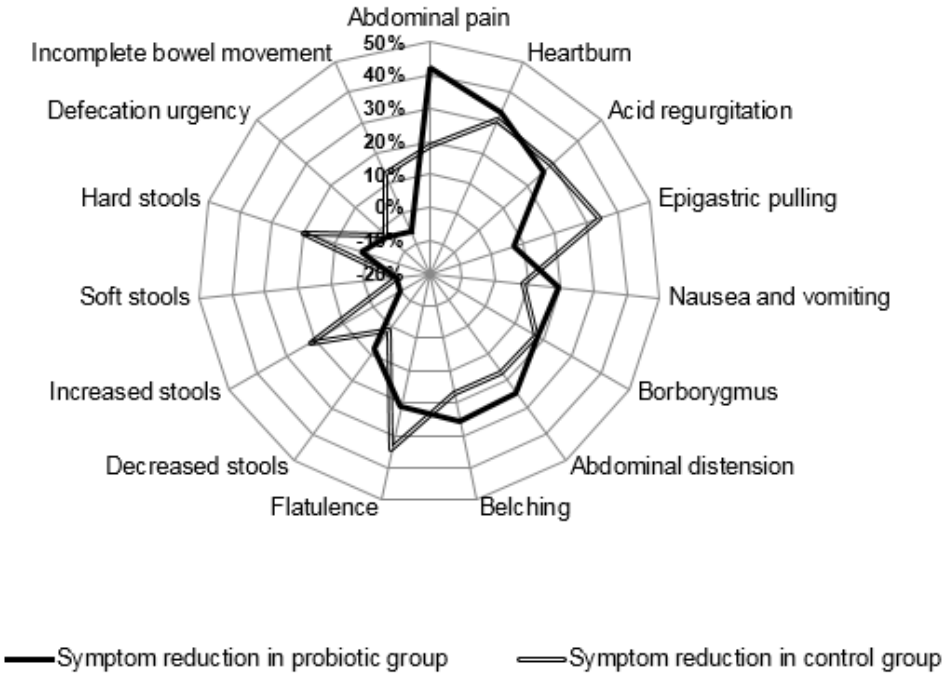


Fig. 3. Assessing the proportion of patients with symptoms (as measured by GSRS) and the magnitude of symptom reduction.